THERMAL DESTRUCTION OF CORN FIBER

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Purpose:

This factorial study should demonstrate the effect of the sterilizing temperature in combination with time, pH, and dilution or concentration of pretreated corn fiber on sugar degradation and fermentation performance of the Labatt 1400 yeast.

Background:

Corn fiber is a feedstock derived from the wet milling process. This material is the proposed carbon source for the biomass to ethanol process in the Pilot Demonstration Unit (PDU). Extensive bench scale studies are being performed to provide fermentation guidelines for the PDU. A sterilization method needs to be developed for the bench scale that will not adversely degrade nutrients and form inhibitors of fermentation. Certain conditions affect degradation of media such as sterilization temperature, time at that temperature, pH of media, and concentration of media components.

The first two conditions have been considered by Deindorfer and Humphrey (1959) who developed a design standard for sterilization. This has been referred to as the Del factor which is represented by the term V. The Del factor is the measure of the fractional reduction in viable organism count produced by a certain heat and time regime. The target Del factor for this experiment will be 32.2 as calculated by the equation:

$$\nabla = \ln \frac{Nt}{No}$$

where N_t is the *number* of spores present in the media before sterilization, and N_o is the *number* of spores present in the media after sterilization.

It is assumed that the spores are from *Bacillus stearothermophilus*, $N_t = 10^{12}$ and $N_o = 10^{-3}$. This gives the acceptable level of risk for contamination of one in a thousand according to Deindorfer and Humphrey (1959), Richards (1968), and Banks (1979). The resulting Del factors calculated for each of the temperature profiles will be compared to this target number.

Methods for preventing contaminant growth that do not require autoclaving were considered and qualifying guidelines established. These guidelines were: (1) safety to the user; (2) efficacious for contamination control; and (3) low negative impact on fermentation performance. Nisin met these guidelines so was included in this experiment. Nisin is a polypeptide bacteriocin produced by strains of *Lactococcus lactis* and is widely used as a food preservative (Delves-Broughton 1990). It is effective for controlling

Gram-positive bacteria and other lactic acid bacteria which are often times the contaminating organism responsible for spoilage of ethanol fermentations (Mawson **1993).**

The **wide** range of conditions chosen for the temperature, holding time, pH, and dilution level **was** designed to determine the effects and magnitude of the effects on the fermentation.

Materials and Methods:

The SSFs were carried out at 30 C with 100 ml of media in ***250*** ml baffled shake flasks. The enzyme employed was from **CPN** and is similar to that used in the **PDU . This** enzyme has a reported **enzyme** activity of **77 IFPU/ml** enzyme. The pretreated corn **fiber** was supplied by Amoco Corporation. The organism was *Saccharomyces* ***cerevisiae*** Labatt **1400** strain. The organism and the feedstock was supplied by *Amoco* Corporation.

Table one lists the specific conditions for each flask and the conditions are repeated here for **clarity.** The temperature varied **from 25** to **130 C.** The sterilization holding times were 0, 7, and **15** minutes, the pH levels were 4.50, 5.75, and 7.00, and the percent full strength media concentrations **were** 10, **55,** and **100.** The Del factors calculated for each sterilization temperature profile were 0, **7.5, 47.1,** 102.4, and **283.5.**

The medium was made by mixing 1% CSL (sigma) with corn fiber and adjusting the pH with NaOH (2M) which required 111 ml of NaOH solution for one Kg of corn fiber slurry. The corresponding weight of full strength corn fiber slurry was added to each flask and deionized water was added to bring the total weight to 90 grams allowing for 10% inoculum volume. The Del factor was calculated by a combination of two methods. The first method was developed by Richards which is the rapid method for the design of sterilization cycles (Richards, 1968) and was used to determine the contribution made by the increasing and decreasing slopes the sterilization cycle. The second method (mentioned above) by Deindoerfer and Humphrey (1959) was used to determine the contribution of the holding temperature and time. These methods were applied to the resulting temperature profiles that were recorded for each autoclave run.

As an alternative to high pressure steam sterilization, nisin (Nisaplin; Alpin & Barrett, UK) was also tested. Two of the flasks in this experiment had 1000 IU/g media of nisin added as an inhibitor to bacterial growth with 100% corn fiber and were not autoclaved. Two additional flasks with 100% corn fiber were controls and had no nisaplin added and received no heat and steam sterilization.

Results and Discussion:

In addition to the various temperatures, times, pH, and concentration of media that each flask experienced during autoclaving , Table One also lists the Del factor for each flask as

calculated by the resulting temperature profile. The Del factors ranged from 0, for the flasks that were not autoclaved, to 283 which is approximately nine times the target value of 32.3. In some cases, the flasks were subjected to more extreme sterilization conditions than would normally be necessary to attain sterilization. In light of these harsh conditions, degradation of media might have been expected but this was not the result as seen by the consistent fermentation rates among all of the tests and degradation of the sugars was very small to non-existent as seen on all four graphs. The bars labeled "Raw Media" are the values of the sugar concentrations before autoclaving and the remaining bars represent the values of the sugar concentrations after autoclaving.

Graph One represents the relative percentage of soluble oligomeric glucose, xylose, and arabinose to the total amount of that soluble sugar present at the initial sampling time. Mannose and galactose were not included because they were not detectable at very small amounts or analysis was unreliable. No shift in the relative amounts of sugars is noted in comparing before to after the autoclaving treatment. This would imply there is no significant loss or hydrolysis of sugars during autoclaving. The samples containing 10% media concentration (flasks 1,4, and 5) appear to be inconsistent with the rest of the field and I suspect that this originates from analysis error. A similar problem is seen with the samples for oligomeric arabinose.

Graph Two depicts the initial and final concentration of oligomeric and monomeric glucose along with cellobiose. While the graph shows that most all of the monomeric

glucose is fermented after 168 hours of SSF, it can be seen there is little reduction of oligomeric glucose throughout the fermentation. This may indicate the necessity of an enzyme to help break down the oligomers into the monomeric form. Most of the cellobiose is utilized in the flasks containing the lower concentrations of media but only half of the cellobiose was utilized in the flasks containing 100% media which may be due to the cellulase enzyme losing its effectiveness over time. There is also some residual monomeric glucose (about 1g/l) left in the flasks containing 100% media. I suspect that this is due to build up of some inhibitory product to fermentation or a nutrient limitation (possibly nitrogen). The presence of monomeric glucose and the continued production of ethanol suggests that the cellulase enzyme and the yeast were still active at the end of the fermentation. The viability of the yeast increased over the course of the 168 hour fermentation which was demonstrated by an increase of 10 CFUs/ml which further demonstrates the ability of the yeast to utilize the glucose present.

Graph Three and Four show the ratios of oligomeric to monomeric xylose and arabinose respectively. There is no observable changes in the ratios from start to finish of autoclaving and over the course of fermentation. This lack of spontaneous equilibrium may need to be remedied by addition of enzymes capable of hydrolyzing these oligomers. The theoretical yield of ethanol as shown in Graph Five ranged from about 55% to 68% and in consideration of the inherent error present in analysis, no observable difference between the flasks and conditions were noted.

The effectiveness of the sterilization methods employed (autoclaving and nisaplin) failed to show any improvements in the elimination of contaminants because of a lack of contaminants in the control flasks. There was also no significant level of contamination in any of the flasks tested. This could be due to the high temperature, low pH pretreatment and the subsequent storage of the pretreated material at a low pH (about pH≅1.4). Although no significant contamination was observed in any of the flasks, the lack of control over the exposure of the possible subsequent pretreated material to spore forming and/or acidophilic contaminants necessitates my recommendation for the continued practice of high pressure steam sterilization.

Conclusions:

The results of this experiment have answered many of the questions asked at the onset of this experiment. This experiment has shown that there is no discernible differences in the fermentation performance of any set of conditions tested. There was also no reduction or change in the oligomeric to monomeric carbohydrate ratio other than that attributed to fermentation of glucose. The fact that the fermentation progressed in all of the flasks at a similar rate shows that no inhibition products were formed during autoclaving. The effectiveness of steam sterilization and nisin have not been demonstrated in this experiment due to the lack of contamination in the control. It may be possible to eliminate additional sterilization after the pretreatment step but in consideration of the consequences

of contamination it would be prudent to continue autoclaving pending further testing. The exact reasons for the glucose not being fully fermented is not clear but could be due to either a nutrient limitation, inhibitory product formation during fermentation, or a combination of both and would be grounds for additional investigation of these areas.

Bibliography:

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Delves-Broughton, J. Food ***Technology*** **44, 100-1** 17 *(1990).*

Mawson, A.J. & Costar, K. ***Letters in Applied Microbiology*** 17, **256-258** (1993).

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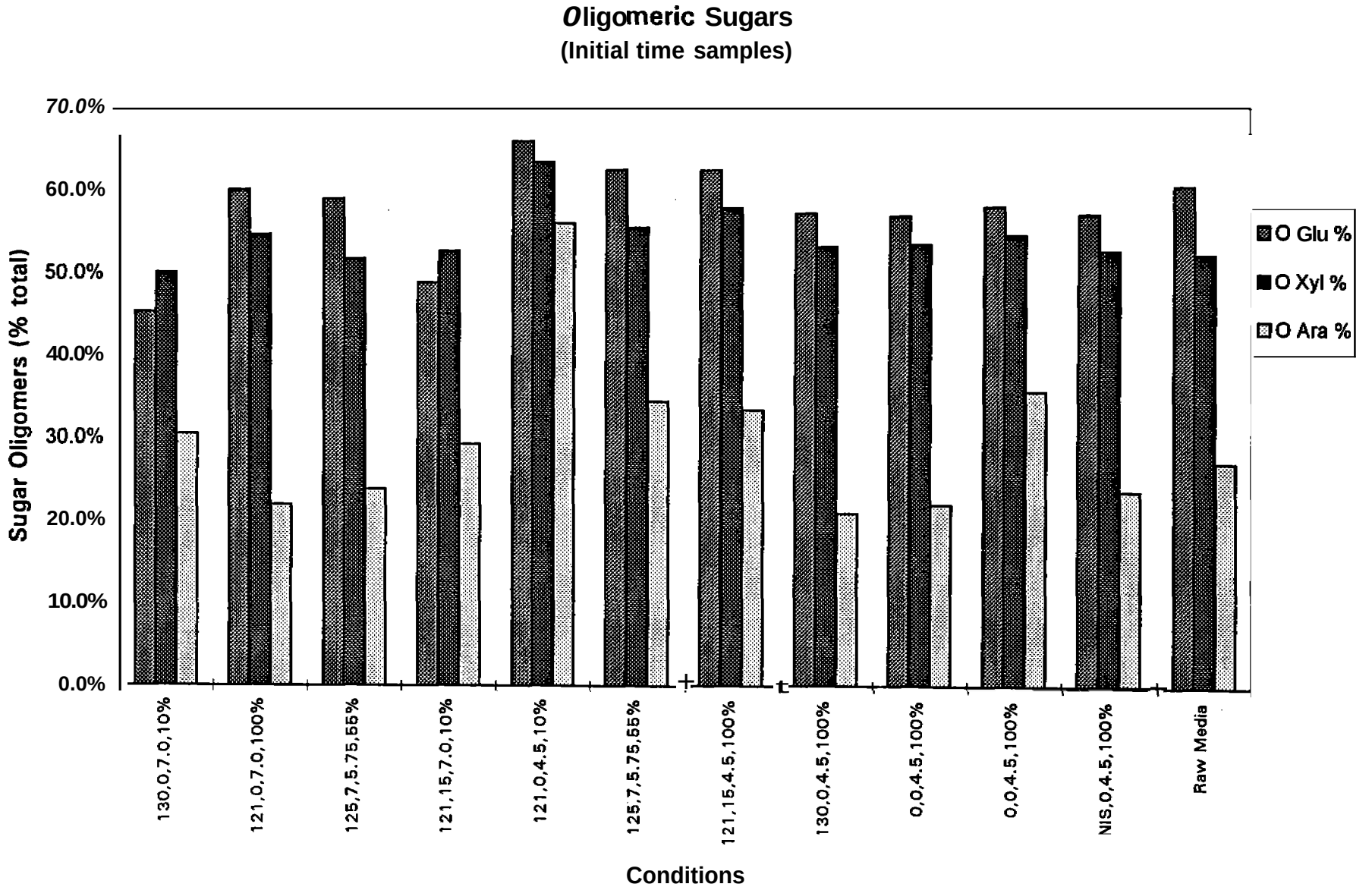
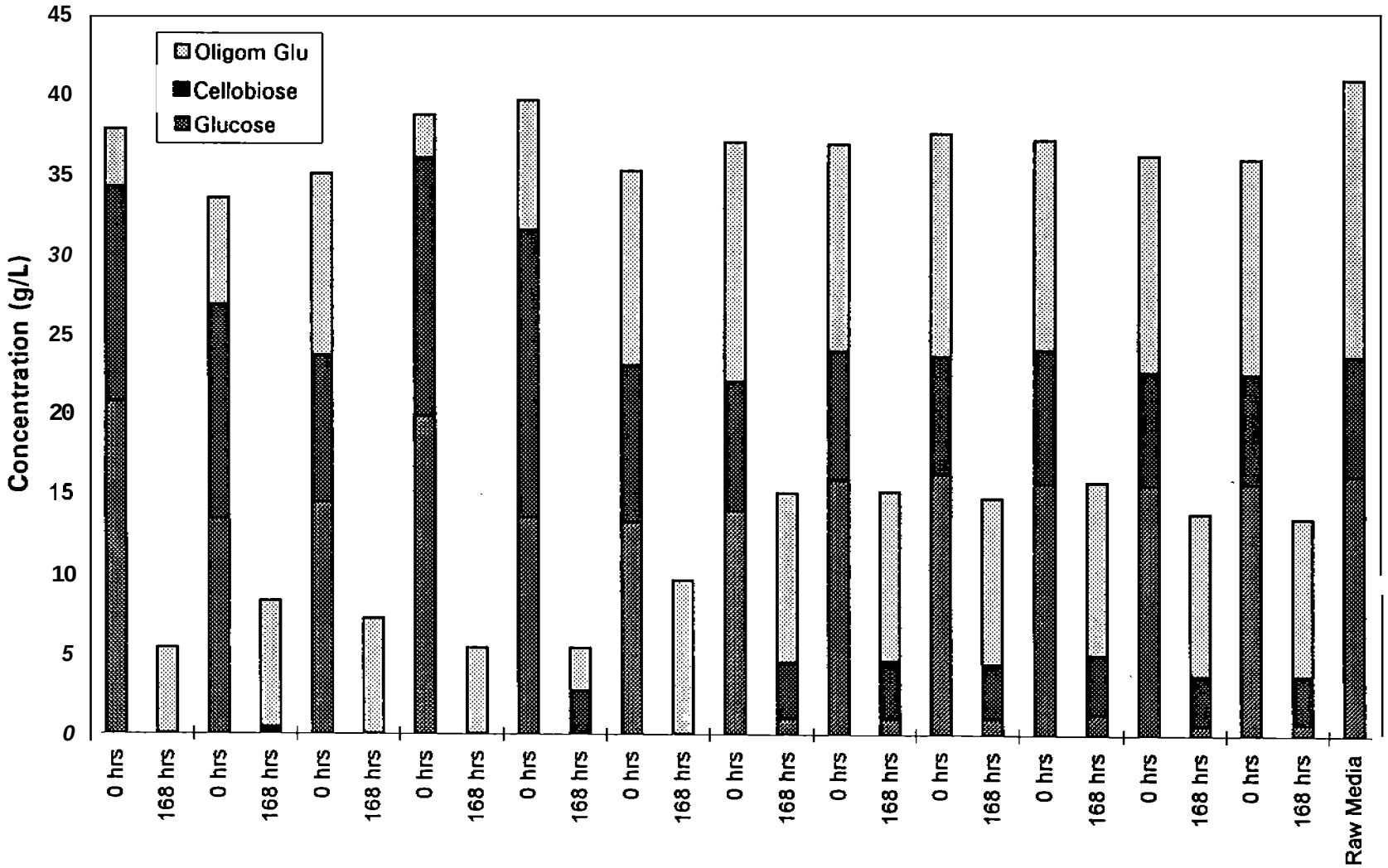
Conditions

	Temp.	Time	pH	Dilution	Del
	degrees C	minutes		% Full	
Flask #1	130	0	7	10	283.5
Flask #2	121	0	7	100	7.5
Flask #3	125	7	5.75	55	102.4
Flask #4	121	15	7	10	47.1
Flask #5	121	0	4.5	10	7.5
Flask #6	1251	7	5.75	55	102.4
Flask #7	121	15	4.5	100	47.1
Flask #8	130	0	4.5	100	283.5
Flask #9	0	0	4.5	100	C
Flask #10	0	0	4.5	100	C
Flask #11	0	0	4.5	100	C
Flask #12	0	0	4.5	100	C

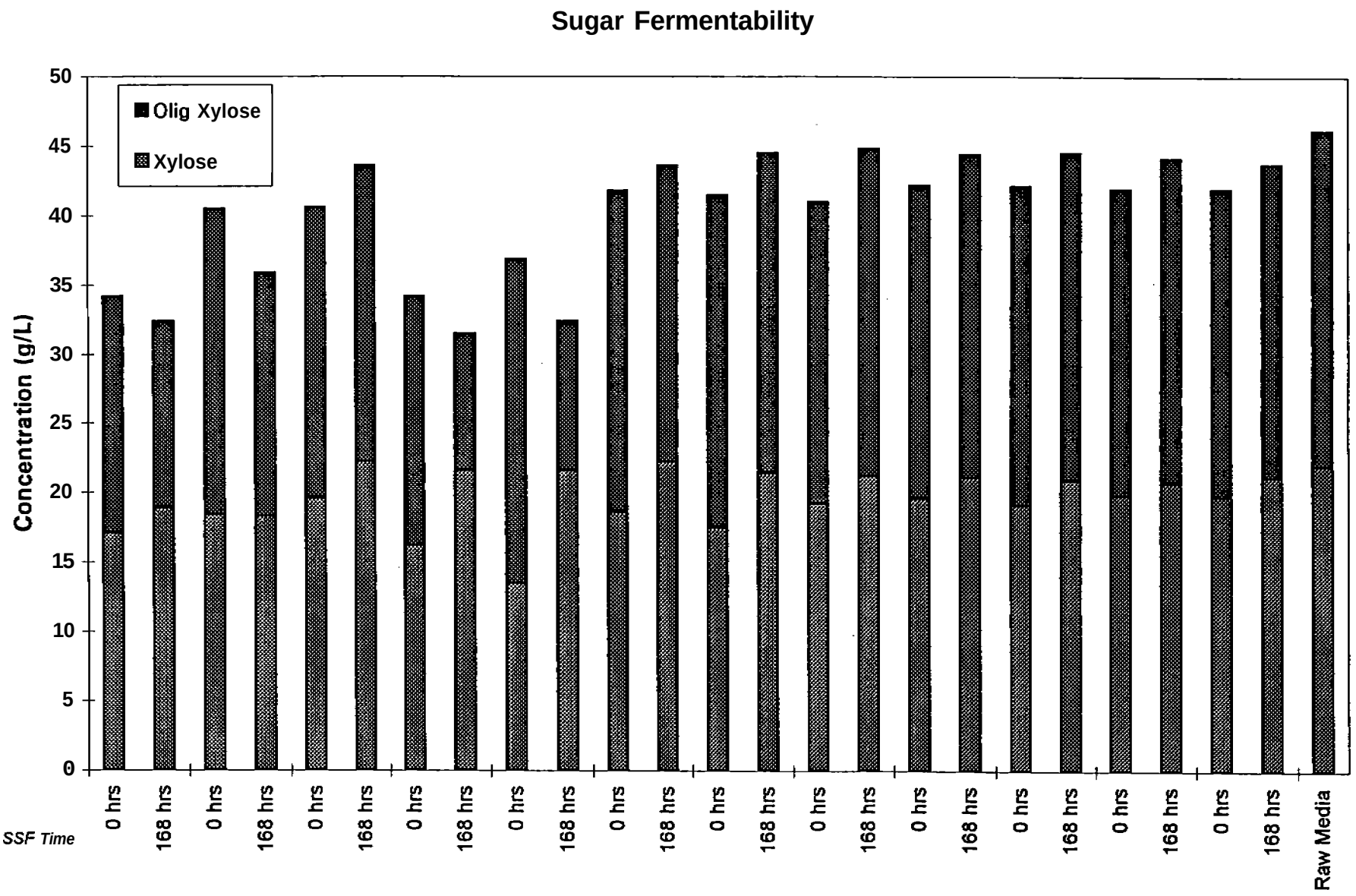

Oligomeric Sugars
(Initial time samples)
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Sugar Oligomers (% total)
O Glu %
O Xyl %
O Ara %
130,0,7.0,10%
121,0,7.0,100%
125,7,5.75,55%
121,15,7.0,10%
121,0,4.5,10%
125,7,5.75,55%
121,15,4.5,100%
130,0,4.5,100%
0,0,4.5,100%
0,0,4.5,100%
NIS,0,4.5,100%
Raw Media
Conditions

G3-FermGlucose

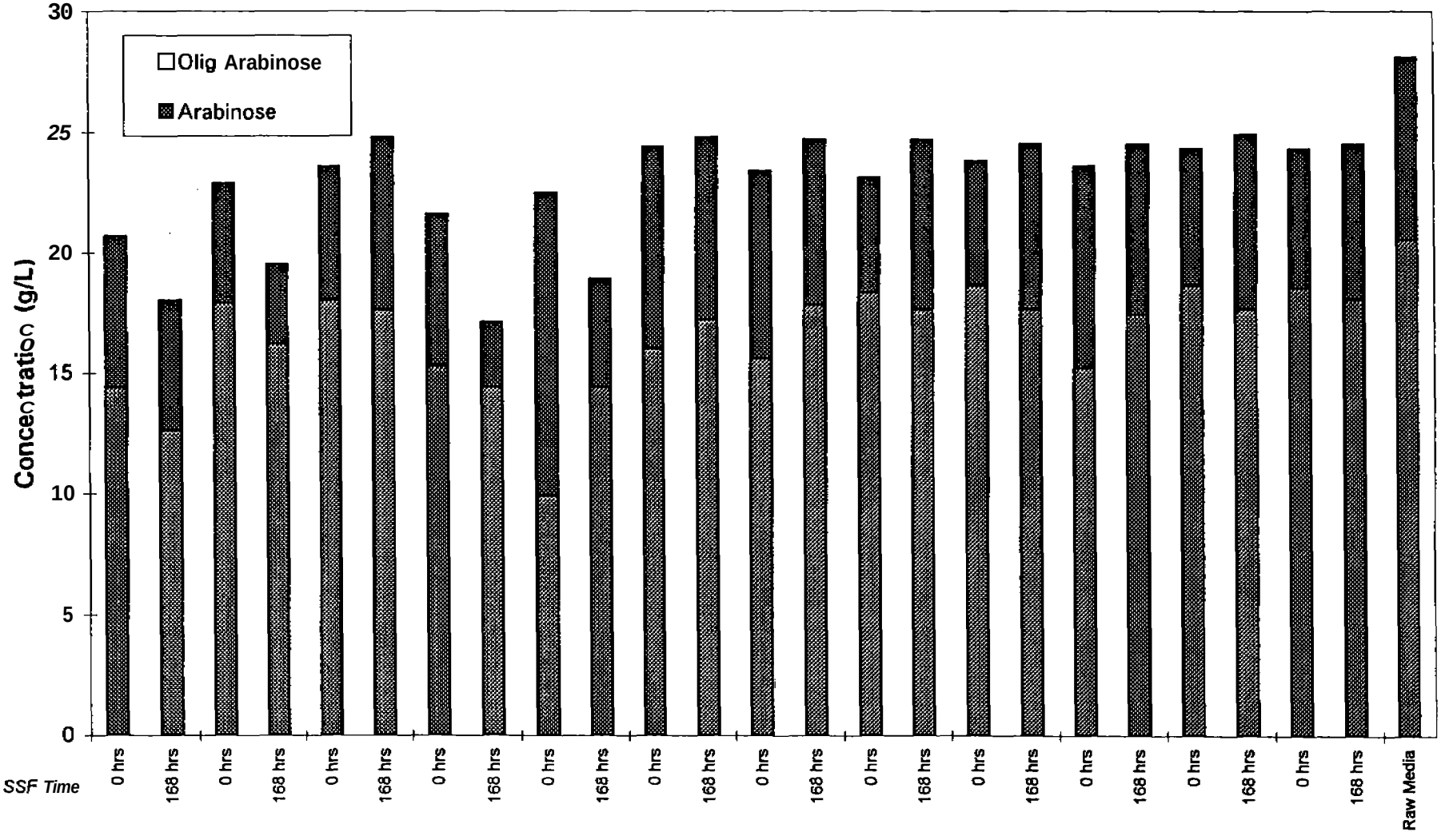
Sugar Fermentability

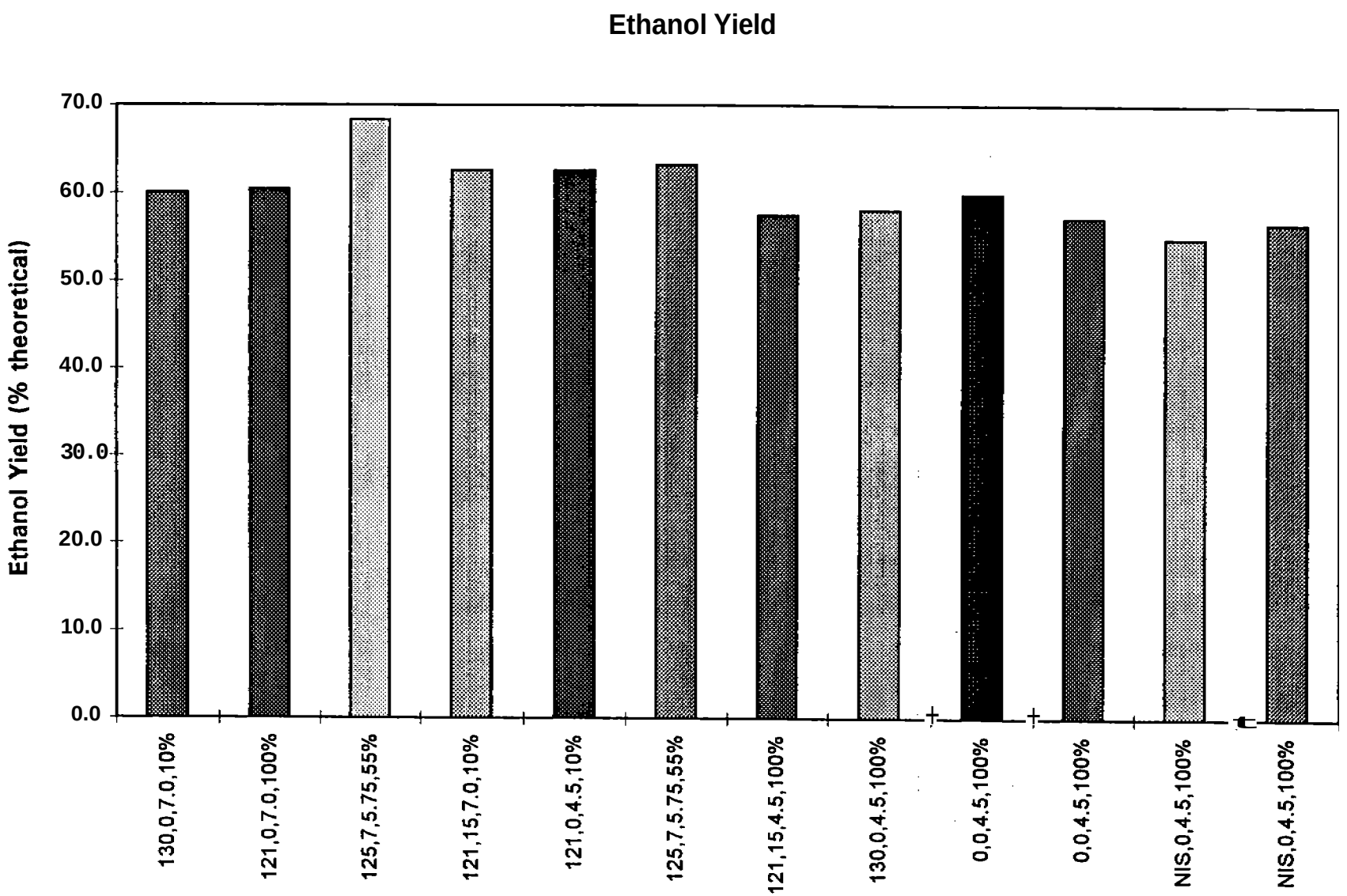


G4-FermXylose



Sugar Fermentability




Ethanol Yield
Ethanol Yield (% theoretical)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
130,0,7.0,10%
121,0,7.0,100%
125,7,5.75,55%
121,15,7.0,10%
121,0,4.5,10%
125,7,5.75,55%
121,15,4.5,100%
130,0,4.5,100%
0,0,4.5,100%
0,0,4.5,100%
NIS,0,4.5,100%
NIS,0,4.5,100%